

Chromosomes of Seven Species of Indian Sphingid Moths

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Abstract. Chromosome cytology of seven species of sphingid moths have revealed the haploid chromosome number as $n=29$ in each of *Acherontia styx*, *Cephonodes hylas*, *Deilephila nerii*, *Macroglossum bombylans*, *Macroglossum gyrans* and *Theretra oldenlandiae* and $n=28$ in *Rhyncolaba acteus*. Peculiar lagging anaphasic movement of two of the homologues of a bivalent (possibly the XX sex-chromosome pair) during their poleward separation has been observed in three out of the seven species examined.

Introduction

Although intense investigations on the chromosome cytology of Lepidoptera have been carried out in different parts of the world, work in India is almost negligible (Gupta, 1964; Saitoh and Abe, 1969, 1970a, 1970b; Rishi, 1973; Nayak, 1975). The present communication deals with karyological study of the male meiotic cycle of seven species of moths belonging to family Sphingidae. Five* are new to cytology. This information may be of great help in providing additional information for an understanding of cytotaxonomic situation of the group.

Material and Methods

Larvae of seven species of sphingid moths were collected from their respective host plants (Table 1) and were reared in cages. The fifth instar larvae and early pupae were found suitable for chromosomal investigation. Testes were dissected out and fixed overnight in 1:3 acetic alcohol. Permanent squash and smear preparations of the material were made following the technique of Smith (1943) and slides were stained in Heidenhains Iron Haematoxylin. Slides were examined under a Meopta Binocular Research Microscope and good metaphase stages were drawn using a 100X oil immersion objective and 15X ocular with a camera lucida. Some of the stages were photographed.

TABLE 1

| Name of Species | Host plant | 2n | n |
|---|-----------------------------|----|----|
| <i>Acherontia styx</i> Westw. | <i>Sesamum indicum</i> | 58 | 29 |
| <i>Cephonodes hylas</i> Linn. | <i>Gardenia</i> sp. | 58 | 29 |
| <i>Deilephila (Daphnis) nerii</i> Linn. | <i>Oleand</i> sp. | 58 | 29 |
| <i>Macroglossum bombylans</i> Bois. | <i>Paedera</i> sp. | 58 | 29 |
| <i>Macroglossum gyrans</i> Walk. | Unidentified | 58 | 29 |
| <i>Rhyncolaba acteus</i> Cram. | <i>Impatiens</i> sp. | 56 | 28 |
| <i>Theretra oldenlandiae</i> Fabr. | <i>Colocasia antiquorum</i> | 58 | 29 |

Observations

*Acherontia styx**

The diploid number (2n) at spermatogonial mitosis consisted of 58 small, spherical and dot-like chromosomes in a circular arrangement. In both size and morphology, the chromosomes were almost identical. Sex chromosomes, if any, remained unidentifiable. The meiotic prophase chromosomes in zygotene and pachytene stages were elongated and thread-like paired bodies, almost half that of the number of spermatogonial metaphase chromosomes. Their exact number, however, was not countable. The pachytene bivalents were faintly stained and appeared to be shorter and thicker, providing evidence of a lengthwise pairing of homologous chromosomes. However, the exact nature and position of chiasmata upon them was not clear. The early diplotene appeared indiscrete. The chromosomes became more and more condensed and stainable as they passed through diakinesis to metaphase I. The mid and late diakinetid bivalents had an uneven distribution in the nucleus and presented various chiasma bearing shapes like V, dumbbell, cross, rod and ring suggesting the positive occurrence of chiasmata. The metaphase I bivalents were at maximum state of condensation and were oval in shape. In most of the diakinetid and metaphase I plates, 29 bivalents were encountered. Deviation from this number was also noticed in many metaphase I cells. Some of the elements appeared relatively smaller and thus might be univalents formed by early resolution of some bivalents. A peculiar anaphase behaviour was recorded in certain anaphase I plates where two of the separating elements, probably the homologues of a bivalent, still occupied a position in the equatorial plate during their poleward movement when all others had reached the poles. The lagging anaphasic movement is characteristic of sex-chromosomes and possibly this pair

constituted the XX-sex chromosome pair. Metaphase II presented 29 dot shaped univalent chromosomes which is in conformity with the haploid chromosome number of the species (Figs. 1 to 4).

*Cephonodes hylas**

2n=58. Polar as well as equatorial views of metaphase I stages showed clearly 29 bivalents. The chromosomes were oval in polar view, but almost dumbbell-shaped with a notch across the middle in equatorial view. Early separation of a bivalent into two distinct elements were observed in 14 out of 171 nuclei examined. Metaphase II showed 29 small univalent chromosomes, establishing the haploid number $n=29$ (Figs. 5 to 7).

*Deilephila nerii**

2n=58. Two of the chromosomes appeared to be positively heteropycnotic. Metaphase I cells showed 29 bivalents, one of which was more deeply stained. In some cells there was an early resolution of a bivalent into univalents. All the bivalents at anaphase I separated synchronously to the poles and sometimes two separating elements, probably the homologues of a bivalent, continued to remain on the equatorial region when all others had nearly reached the poles. Metaphase II cells showed 29 univalents confirming the haploid number, $n=29$ (Figs. 8 to 10).

*Macroglossum bombylans**

2n=58. Metaphase I cells showed 29 bivalents. Though anaphase I was normal, in a good number of dividing cells, lagging behaviour of a bivalent was significant. Metaphase II cells showed 29 univalent chromosomes (Figs. 11 to 13).

*Macroglossum gyrans**

2n=58. Metaphase I cells showed 29 bivalents. In most cells one bivalent was deeply stained while occasionally an early separation of a bivalent to its components was seen. Anaphase I was normal. Metaphase II cells showed 29 univalents establishing the haploid number as $n=29$ (Figs. 14 to 16).

Rhyncolaba acteus

2n=56. Metaphase I cells showed 28 bivalents. Metaphase II showed 28 univalents. The present investigation, while recording a deviation in the chromosome number ($n=28$) of the species from the modal haploid number ($n=29$) for the family Sphingidae, also records a difference from the number ($n=29$) already recorded for this species by Rishi (1975) (Figs. 17 to 19).

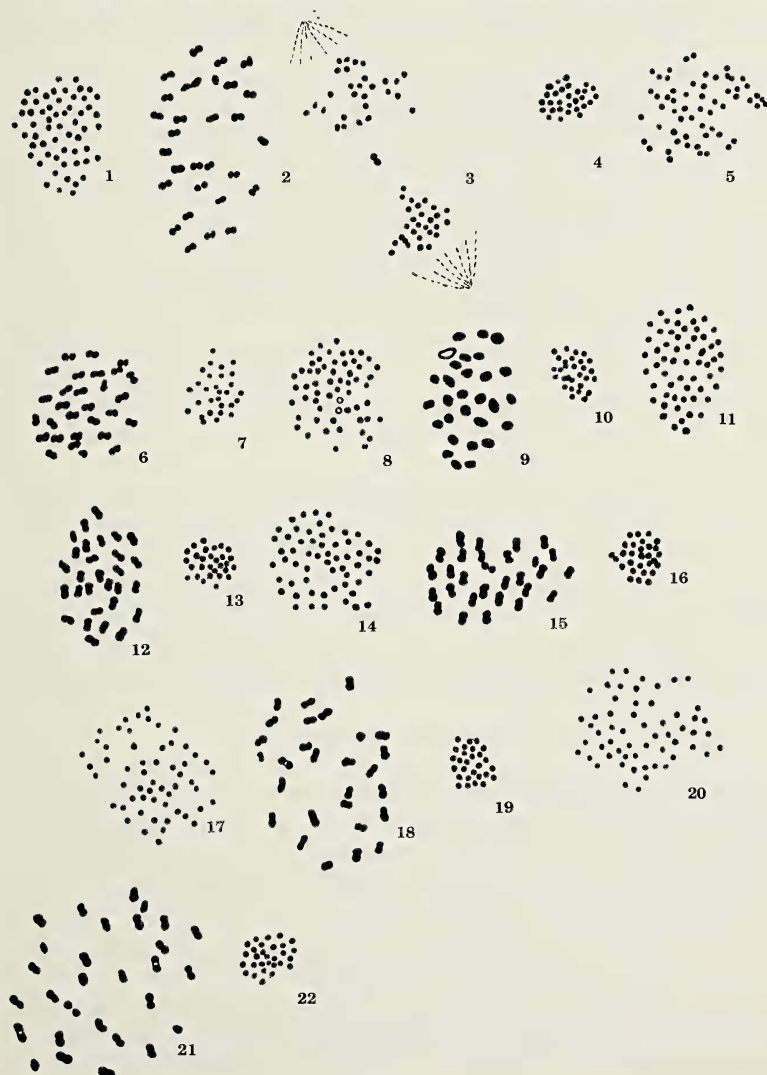
Theretra oidenlandiae

2n=58. Metaphase I cells contained 29 bivalents. Precocious separation of a single bivalent into two univalents was apparent in many plates. Metaphase II showed 29 univalents. The haploid number $n=29$ deter-

mined for this species is at variance with $n=31$ reported by Gupta (1964) (Figs. 20 to 22).

Discussion

The remarkable uniformity, except for minor details, in morphology and behaviour of lepidopteran chromosomes during the meiotic cycle have been well documented (Seiler, 1914; Beliajeff, 1930; Federley, 1928; Lorkovic, 1941; Gupta, 1964; Suomalainen, 1969b, 1971; Rishi, 1975;



Nayak, 1975). The present investigation agrees with the earlier published data. The chromosomes are minute, homomorphic and isodiametric, presenting a circular distribution in mitotic metaphase stages. In the family Sphingidae, 31 species have been cytologically investigated thus far and the modal haploid number for this family has been established as $n=29$ (Federley, 1928; Beliajeff, 1930; Saitoh, 1959, 1960; Robinson, 1971). The next most frequent numbers for this family are 28 and 27, while other numbers are 31, 33, 49, 52 and 59. The highest chromosome number in the family ($n=59$ in *Langia zenzeroides nawai*) has been reported by Saitoh and Kumagai (1973). It differed from all others in having the haploid number almost double that of the modal number ($n=29$)—a situation showing an indication of a phylogenetic peculiarity of this subspecies. In the present analysis, the haploid chromosome number ascertained in six out of seven Sphingid moths is uniformly 29, in accordance with the modal haploid number for the family. However, in one species, *Rhyncolaba acteus*, the base number has been determined to be less by one, i.e., $n=28$, although at variance with the observations made by Rishi (1973) who reported the chromosome number as $n=29$ for the same species. This deviation in chromosome number may be due to geographical variation. Similarly, a variation in the chromosome counts of *Theretra oldenlandae* has been recorded. While our observations establish the haploid number for this species as $n=29$, that of Gupta (1964) records it as $n=31$. This chromosome polymorphism as well may be due to geographical variation.

Analysis of sex chromosomes in Lepidoptera has been greatly impeded due to the homomorphic nature of the metaphase chromosomes which make the sex chromosomes and autosomes undifferentiable. In Lepidoptera, the female is the heterogametic sex with the XY (ZW) or XO (ZO) sex chromosome mechanism while the male is of the XX (ZZ) type. In other insect orders the males are usually the heterogamous sex. No definite information is available as to the manner in which the switchover from male to female heterogamety occurred in the Lepidoptera during evolution from their presumed mecopteroid ancestors.

A heteropycnotic pair of chromosomes associated with the nucleolus during the spermatogenesis of *Philosamia cynthia* was considered as the sex chromosome pair by Kawaguchi (1937) and earlier by Kurihara (1929). The large bivalent occurring in the chromosome garniture of some species of Lepidoptera has often been considered as the sex bivalent (Bauer, 1943; White, 1973; Suomalainen, 1969b, 1971; Ennis, 1976). Traut and Mosbacher (1968) distinguished the sex chromatin as a distinctly heteropycnotic body in the somatic interphase nuclei in the females of 70 out of 83 species they examined and took it as the Y-chromosome since the presence of such a body was not observed in the corresponding males. In our investigations, which have been confined to the males only, we could

not observe any heteropycnotic element associated with the prophase nucleus during spermatogenesis, nor was there any large bivalent to be attributed a sex chromosome as upheld by Suomalainen (1969). However, during anaphase I in three out of the seven species of Sphingid moths investigated, a peculiar late separation of a pair of chromosomal elements was occasionally observed. The pair remained midway between the groups of separating chromosomes which had almost reached the poles. This pair may be suspected as the XX sex chromosome pair of the males in view of the occurrence of similar lagging behaviour shown by sex chromosomes in other groups of insects.

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